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Do plant mites commonly prefer the underside of leaves?

Masaaki Sudo* & Masahiro Osakabe

Abstract

The adaxial (upper) and abaxial (lower) surfaces of a plant leaf provide heterogeneous habitats for small arthropods with different environmental conditions, such as light, humidity, and surface morphology. As for plant mites, some agricultural pest species and their natural enemies have been observed to favor the abaxial leaf surface, which is considered an adaptation to avoid rain or solar ultraviolet radiation. However, whether such a preference for the leaf underside is a common behavioral trait in mites on wild vegetation remains unknown. The authors conducted a 2-year survey on the foliar mite assemblage found on *Viburnum erosum* var. *punctatum*, a deciduous shrub on which several mite taxa occur throughout the seasons, and 14 sympatric tree or shrub species in secondary broadleaf-forest sites in Kyoto, west-central Japan. We compared adaxial–abaxial surface distributions of mites among mite taxa, seasons, and morphology of host leaves (presence/absence of hairs and domatia). On *V. erosum* var. *punctatum*, seven of 11 distinguished mite taxa were significantly distributed in favor of abaxial leaf surfaces and the trend was seasonally stable, except for Eriophyoidea. Mite assemblages on 15 plant species were significantly biased towards the abaxial leaf surfaces, regardless of surface morphology. Our data suggest that many mite taxa commonly prefer to stay on abaxial leaf surfaces in wild vegetation. Oribatida displayed a relatively neutral distribution, and in Tenuipalpidae, the ratio of eggs collected from the adaxial vs. the abaxial side was significantly higher than the ratio of the motile individuals, implying that some mite taxa exploit adaxial leaf surfaces as habitat.

Keywords Habitat heterogeneity, Adaxial–abaxial distribution, Domatia, Trichome, Behavioral adaptation

Introduction

Many plants have evolved diverse topographies between adaxial (upper) and abaxial (lower) leaf surfaces, e.g., in possession of leaf domatia (O’Dowd and Willson 1989; Nishida 2004), density and shape of hairs (Chien and Sussex 1996), thickness of wax and cuticle (Price 1980), and density of stomata (Gutschick 1999). Such structures influence microclimates and cause differences in temperature and humidity (Grostal and O’Dowd 1994; Gutschick 1999; cf. Weintraub et al. 2007). Additionally, leaves function as protective shields against solar ultraviolet (UV) radiation by accumulating phenolic compounds such as flavonoids (Izaguirre et al. 2007). The heterogeneity in UV radiation leads to differences in abundance of phylloplane fungi between upper and lower leaf surfaces (Newsham et al. 1997). As a result, adaxial and abaxial leaf surfaces offer heterogeneous environments for plant-dwelling arthropods, and such differences in environment may affect the behavior of inhabitants and their (inter-specific) interactions, for instance, foraging efficiency (Krips et al. 1999).

Therefore, the existence of various strategies relevant to direct and indirect effects of the environment are expected in the adaxial-abaxial leaf surface exploitation and distribution patterns of arthropod communities. Nevertheless, even though in the

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literature the effects of structure and/or architecture of foliage on abundance and behavior of arthropods have been addressed (Johnson 1975; Walter 1996), the between-surface distributions were scarcely taken into consideration. This might be due to the vague common knowledge that most arthropods remain on the lower leaf surfaces. Yet, surprisingly few precise data are available of the exploitation and distribution of arthropods between adaxial and abaxial leaf surfaces.

Mite species belonging to various trophic guilds (i.e., herbivore, predator, fungivore or omnivore) are important components of arthropod communities on the foliage of terrestrial plants. Recently, the effects of solar UV radiation on survival and spatial distribution between the upper and lower leaf surfaces have been investigated in herbivorous spider mites (Ohtsuka and Osakabe 2009; Suzuki et al. 2009; Sakai and Osakabe 2010) and predaceous phytoseiid mites (Onzo et al. 2010). ‘Reverse gravity’ experienced by inhabitants on the upper or lower leaf surfaces was also suggested to affect the distribution of a spider mite, i.e., mites preferred the leaf surface facing downward, regardless whether this was the morphological adaxial or abaxial side (Li and Margolies 1991). Domatia and dense hairs protect mites from low humidity and from inter- and/or intraguild predators (Grostal and O’Dowd 1994; Krips et al. 1999; Norton et al. 2001; Kasai et al. 2005). Plants bearing such foliar structures harbor more predaceous and fungivorous mites, such as Phytoseiidae or Tydeoidea, relative to herbivorous mites (O’Dowd and Willson 1989; O’Dowd and Pemberton 1998; Sudo et al. 2010). However, high and dense leaf hairs are also effective in retaining spider mites and, therefore, might reduce both the direct and indirect effects of predatory mites such as phytoseiids (Oku et al. 2006). Generalist phytoseiid mite species, of the so-called Type III, are usually found in sheltered places on the undersides of leaves, e.g., along the midrib, among leaf hairs or in domatia (McMurtry and Croft 1997). This indicates the close relationship between adaptive strategies of plant-dwelling mites and the structure and/or architecture of host leaves. Consequently, the question arose as to whether the variation of leaf structures influences the distribution of mites and/or their strategy of resource exploitation between adaxial and abaxial leaf surfaces.

Sudo et al. (2010) reported that in a temperate forest, woody plant species such as *Viburnum erosum* Thunb. var. *punctatum* Franch. et Sav. (Adoxaceae; hereafter abbreviated as VEP) with domatia and/or trichomes harbor more mites than those without such microstructures. Major mite taxa found on VEP were the predaceous family Phytoseiidae, the herbivorous superfamily Eriophyoidea, the fungivorous family Winterschmidtidae, and the fungivorous (Walter and Proctor 1999) superfamily Tydeoidea (Sudo et al. 2010). In this study, we inspected the effects of leaf morphology on the adaxial–abaxial leaf-surface distribution of mites on VEP and other sympatric trees or shrub species in the temperate forests in Kyoto, west-central Japan. We also studied the seasonal and diel change in the intra-leaf distribution of mites on VEP.

Materials and methods

Study sites and plants

For the comparison of intra-leaf mite distribution between plants with different leaf-surface morphologies and for the analyses of seasonal changes in intra-leaf distribution of mite taxa on VEP, we sampled mites from two sites in secondary broadleaf forests on the outskirts of Kyoto, Japan: Uryu-yama (35°2'8"N, 135°47'53"E, 110–150 m a.s.l.) and Iwakura (35°5'28"N, 135°46'42"E, 150–160 m a.s.l.). The study site in the Uryu-yama was located along a 50-m-long trail on a spur, and *Eurya japonica* Thunb. (Theaceae), *Rhododendron macrosepalum* Maxim. (Ericaceae), and VEP dominated the understory in a forest whose canopy was dominated by *Quercus serrata* Thunb. and *Q. glauca* Thunb. (Fagaceae). The study site in Iwakura was located alongside a 100-m-long unshaded road, and VEP and *Sasa* sp. (Poaceae) dominated the

understory in a forest whose canopy was dominated by *Pinus densiflora* Siebold et Zucc. (Pinaceae) and *Q. serrata*.

Samplings for VEP were conducted once a month from April to November 2007 and replicated from June to November 2008. Eleven VEP shrubs were randomly selected in each study site in March 2007. However, because six VEP shrubs were felled in the course of the 2-year survey, six neighboring VEP shrubs were newly selected for subsequent samplings. For the plant species other than VEP, sampling was conducted four times (August 2007, June 2008, August 2008, and October 2008). In early August 2007, 29 trees or shrubs of 14 woody species were selected from the vicinity of the target VEP shrubs in Uryu-yama; by June 2008, nine of the selected plants were lost due to felling or blight. Consequently, 20 individuals were added to normalize the number of replication among the categories of leaf surface morphology (see the section “Definition of leaf-surface morphology”), and 148 individual plants in total other than VEP were used for the four sampling periods (Table 1). Foliar acarofauna and their seasonal fluctuations at these two study sites during 2007-2008, with details of the plants used, have been reported by Sudo et al. (2010).

We additionally conducted a survey on the diel dynamics of intra-leaf mite distribution on nine VEP individuals in Iwakura on 31-Oct-2009.

Sampling and counting of mites

Sampling was conducted during daytime but never on rainy days or on days when it had rained in the preceding 24 h. For the sampling from VEP, we collected six leaves from branches 0.5–2.0 m high of each plant, in which each leaf was taken from separate branches chosen randomly within a plant. Leaves with chlorosis symptoms were excluded from the sampling. Sampled leaves were immediately placed in a plastic bag, put vertically in an expanding file, and brought to the laboratory. For the sampling from the plants other than VEP in Uryu-yama, we randomly collected 10 (in August 2007) or six (in June 2008, August 2008, and October 2008) leaves from branches 0.4–3.0 m high of each plant. We conducted sampling from the selected non-VEP plants within 3–4 days after the VEP sampling. Each leaf was taken from separate branches chosen randomly within a plant. From *Acanthopanax sciadophylloides* Franch. et Sav. and *Evodiopanax innovans* Nakai (both Araliaceae), which possessed palmate and ternate leaves, respectively, we sampled only terminal leaflets.

The leaves were placed in a freezer (–10 °C) for >2 days before counting mites. All mites except eggs were collected and identified to the family and/or suborder level under a stereomicroscope. On each plant species, mites that were expected to belong to a species newly found were mounted on microscope slides using Hoyer’s medium. We identified the specimens to the species level using the identification keys of Ehara (1980, 1993), Ehara and Gotoh (2009), and Okabe (2006). We recorded the number of mites from each leaf side (adaxial or abaxial leaf surfaces; abaxial data contained both individuals on leaf blades and inside domatia) for each plant species. Sexes and developmental stages of motile mites were not distinguished. Mites for which the families or higher taxa were not determined were excluded from analyses (45 individuals from VEPs and 88 from other plants; thus, sample sizes differed from those in Sudo et al. 2010). Eggs of Phytoseiidae and Tenuipalpidae on VEP were also counted, in addition to the motile mites. We attempted to identify eggs of Phytoseiidae and Tenuipalpidae according to Ehara (1993) and estimated them from the occurrence of adult individuals on the same leaf. Eggs of other mite taxa were not counted due to the difficulty of identification from external morphology.

Comparison of intra-leaf mite distribution between plants

Definition of leaf-surface morphology

Based on presence/absence of hairs and domatia, we classified target plant species into the following six categories (Table 1): “U+L+D+” (with hairs on both the upper and lower sides, and with leaf domatia), “U+L+D−” (hairs on upper and lower surface, no domatia), “U−L+D+” (hairs on lower side only, domatia in abaxial vein axils), “U−L+D−” (hairs only on lower surface), “U−L−D+” (no hairs, domatia present), and “U−L−D−” (no hairs, no domatia). No plants were observed with only upper-side hairs and domatia (“U+L−D+”), or with only upper-side hairs (“U+L−D−”). Hair shape and characteristics were stellate in VEP, scale in *Elaeagnus pungens* Thunb. (Elaeagnaceae), glandular trichome in *R. macrosepalum*, and short and non-glandular in the other plants. All plant species classified into any of the ‘D+’ classes had a bunch of hairs hanging over the vein axil, which is defined as a ‘tuft’-type domatium by O'Dowd and Willson (1989), and Nishida (2004); *A. sciadophylloides* and *E. innovans* had cavities in addition to hair tufts in their vein axils, thus a mixture of tuft- and ‘pocket’-type domatia (O'Dowd and Willson 1989).

Table 1 Subject plant species used for comparison of intra-leaf distribution of foliar mites between leaf morphologies. Plant species were divided by the presence (+) or absence (−) of hairs on the upper surface (U), hairs on the lower surface (L), and of leaf domatia (D). VEP samples contained only leaves from sampling periods of August 2007, June 2008, August 2008, and October 2008.

Cat. of leaf morphology	Plant species (families)	Total no. of leaves
U+ L+ D+	<i>Viburnum erosum</i> var. <i>punctatum</i> (VEP) (Adoxaceae)	270
U+ L+ D−	<i>Rhododendron macrosepalum</i> , <i>R. obtusum</i> var. <i>kaempferi</i> (Ericaceae)	176
U− L+ D+	<i>Lyonia ovalifolia</i> (Ericaceae)	74
U− L+ D−	<i>Quercus glauca</i> (Fagaceae), <i>Pourthiaea villosa</i> var. <i>laevis</i> (Rosaceae), <i>Elaeagnus pungens</i> (Elaeagnaceae)	160
U− L− D+	<i>Acer palmatum</i> (Aceraceae), <i>Acanthopanax sciadophylloides</i> (Araliaceae), <i>Evodiopanax innovans</i> (Araliaceae), <i>Abelia serrata</i> (Caprifoliaceae)	278
U− L− D−	<i>Lindera umbellata</i> (Lauraceae), <i>Eurya japonica</i> (Theaceae), <i>Vaccinium hirtum</i> (Ericaceae), <i>Osmanthus heterophyllus</i> (Oleaceae)	316

Statistical analyses

For comparisons of adaxial–abaxial distribution of mites among mite taxa and/or categories of plant species with different leaf-morphology traits, we constructed log-linear models and evaluated them by both Akaike's information criterion (AIC) and the Bayesian information criterion (BIC). R version 2.10.1 (R Development Core Team 2009) and the ‘loglm’ module of R (in package MASS by Venables and Ripley 2002) were used for construction and selection of these models. The categories with no mites collected were regarded as sampling zeros, and we added 0.001 to the observed frequencies of all counted data prior to model construction.

To detect the difference in intra-leaf mite distribution between plants with different leaf-surface morphology, we constructed log-linear models containing “leaf” (six categories of leaf-surface morphology), “mite” (seven distinguished mite

taxa: see below), and “side” (adaxial or abaxial) as explanatory variables to explain the observed frequency (Table 2). Thus, the constructed log-linear model represented a $6 \times 7 \times 2$ contingency table. According to Sudo et al. (2010), we used the following seven categories of mite taxa for model construction: Winterschmidtidae, Tydeoidea, Eriophyoidea, Phytoseiidae, Stigmaeidae, Oribatida, and a mixture of minor taxa (Anystidae, Cunaxidae, Tetranychidae, Tenuipalpidae, and Ixodida; see Sudo et al. 2010 for the numbers of collected mite specimens). We constructed nine models with all possible combinations of multi-way interactions and calculated the AIC and BIC for each combination. Data from the four sampling periods were combined prior to analysis, as the composition of identified mite taxa in fact did not change with seasons (Sudo et al. 2010).

To evaluate the effect of each categorical factor in selected models on the adaxial–abaxial distribution, we used R×C Fisher’s exact test for independence and then used paired Fisher’s exact test with Holm–Bonferroni correction. This was due to sample sizes that were extremely different among categories (several to tens of thousands), and other comparison methods, such as the chi-square test, were inadequate.

Table 2 Multispecies log-linear models for factors “leaf” (six categories of leaf-surface morphology) and “mite” (seven distinguished taxa), that affect intra-leaf distribution of mites (“side”). Underlined figures in columns “AIC” and “BIC” signify the smallest value (i.e., the model supported by the criterion). The left side of each model formula is the observed frequency of mites

Model	df	X_L^2	AIC	BIC
leaf + mite + side + leaf × mite + leaf × side + mite × side + leaf × mite × side	84	0.00	<u>168.00</u>	760.69
leaf + mite + side + leaf × mite + leaf × side + mite × side	54	83.64	171.64	552.65
leaf + mite + side + leaf × side + mite × side	24	1990.79	2038.79	2208.13
leaf + mite + side + leaf × mite + mite × side	49	72.54	170.54	<u>516.28</u>
leaf + mite + side + leaf × mite + leaf × side	48	516.36	612.36	951.04
leaf + mite + side + leaf × mite	43	557.39	643.39	946.79
leaf + mite + side + leaf × side	18	2475.64	2511.64	2638.64
leaf + mite + side + mite × side	19	2031.82	2069.82	2203.88
leaf + mite + side	13	2516.66	2542.66	2634.39

Seasonal change in intra-leaf distribution of mite taxa on VEP

Over the 14 sampling periods (i.e., once a month from April to November 2007 and June to November 2008), we sampled leaves from 158 VEP shrubs in total for the Uryu-yama site (sample size of each period varied from 8 to 12) and from 148 VEPs in total for the Iwakura site (sample size of each period varied from 10 to 11).

To evaluate whether mite taxa and/or seasons affect the adaxial–abaxial distribution on VEP leaves, we constructed log-linear models and evaluated them in the same way as the multispecies comparison mentioned above. The models contained “season” (14 months, no nest structure of duplicated months in 2 years), “mite” (nine mite taxa: see below), and “side” (adaxial or abaxial) as explanatory variables to explain the observed frequency (Table 3). Thus, the constructed log-linear model represented a $14 \times 9 \times 2$ contingency table. As in Sudo et al. (2010), we used the following nine mite taxa for model construction: Winterschmidtidae, Tydeoidea, Eriophyoidea, Phytoseiidae, Tenuipalpidae, Tetranychidae, Oribatida, Stigmaeidae, and a mixture of minor taxa (Anystidae, Cunaxidae, and Ascidae). The effects of each categorical

factor in the selected model on the adaxial–abaxial distribution were evaluated by R×C Fisher’s exact test for independence and paired Fisher’s exact test with Holm–Bonferroni correction.

Table 3 Log-linear models for factors “season” (14 months) and “mite” (nine distinguished taxa), that affect the intra-leaf distribution of mites (“side”) on VEP at the Uryu-yama or Iwakura sites. Underlined figures in columns “AIC” and “BIC” signify the smallest value in each site (i.e., the model supported by the criterion). Only the saturated model and supported model(s) in each site are shown. The left side of each model formula is the observed frequency of mites

Model	df	X_L^2	AIC	BIC
<i>Uryu-yama</i>				
season + mite + side + season × mite + season × side + mite × side + season × mite × side	252	0.00	504.00	2530.10
season + mite + side + season × mite + season × side + mite × side	148	112.05	<u>408.05</u>	1597.98
season + mite + side + season × mite + mite × side	135	224.90	494.90	<u>1580.31</u>
<i>Iwakura</i>				
season + mite + side + season × mite + season × side + mite × side + season × mite × side	252	0.00	504.00	2480.50
season + mite + side + season × mite + season × side + mite × side	148	109.39	<u>405.39</u>	<u>1566.19</u>

Survey on the diel dynamics of intra-leaf mite distribution on VEP

To investigate diurnal changes in the intra-leaf distribution of mites on VEP, we compared mite distribution between day- and nighttime. We sampled mites from nine VEP individuals in Iwakura on 31 October 2009. These shrubs were the survivors of the individuals used in the preceding sampling from 2007 to 2008. We sampled twice from the same plants, during the day (13:30–14:30) and at night (23:30–00:10). Approximately 1 h before the daytime sampling, six branches were randomly chosen from each target VEP shrub, and we bound color wires on the basal parts of the branches as a mark. Each branch had at least two intact leaves. We cut out three of the six branches from each shrub with scissors at the daytime sampling and the night sampling. To immobilize mites, the leaf surfaces of each branch of the shrub were immediately sprayed with an art fixative (Spray Fixative No. 600; Holbein Art Materials, Osaka, Japan) from a distance of 15 cm for 2 s, air-dried for 30 s, and then brought to the laboratory.

We collected mites from two apical leaves on each sampled branch. Due to technical requirements, mites were counted twice. Leaves were defrosted before the first inspection, and we counted part of the mite population we could recognize through semitransparent milky-white resin on the adaxial and abaxial leaf blades; we then classified them into families under the stereomicroscope (20×). These mites were removed from leaves after the first inspection. Leaves were then stirred gently in 99% acetone for 40 s and soaked for 60 s in 70% ethanol. Resin was removed by acetone treatment, but acetone hardened the lamina. Therefore, we treated the leaves with ethanol to soften them for the second inspection. After 5 min of air-drying, we re-counted mites of all motile forms on the adaxial and abaxial blades and domatia, that we were not able to find in the first inspection; they were classified into families under the stereomicroscope (30×) and added to the first inspection samples. At the end, we recorded the number of mites that had dropped into the media (both acetone and ethanol fraction), which were not determined by intra-leaf location and thus excluded from analyses. The significance of diurnal difference in adaxial–abaxial distribution of each mite taxon was evaluated by Fisher’s exact test.

Results

Comparison of intra-leaf mite distribution between plants

For comparison of the mite distributions among VEP and other plants, we collected 8,568 mite specimens (another 90 unidentified specimens were not included) in 11 mite taxa from 1,274 leaves of 15 plant species during the four sampling periods in Uryu-yama, of which 8,376 mites (97.8%) (95% CI for binomial distribution: 97.4-98.1%) were collected from abaxial leaf surfaces. Four taxa – Winterschmidtidae, Tydeoidea, Eriophyoidea, and Phytoseiidae – dominated the foliar acarine communities. For those four taxa, individuals from the abaxial leaf surfaces accounted for 99.1% (95% CI: 98.8-99.4%), 99.6% (99.3-99.7%), 79.1% (75.5-82.4%), and 93.8% (89.7-96.5%), respectively, and all showed a significantly biased distribution (Exact binomial tests, $P < 0.001$). Of the four major mite taxa, three – Winterschmidtidae, Tydeoidea, and Phytoseiidae – did not show any significant differences in adaxial–abaxial distribution among categories of leaf-surface morphology (Fig. 1; 2×6 Fisher's exact test, $P > 0.05$). The ratio of Eriophyoidea individuals collected from the adaxial side was significantly higher on U–L+D– (with only abaxial hairs, no domatia) than on U–L–D+ (no hairs, only domatia) (Fig. 1; Paired Fisher's exact test with Holm–Bonferroni correction, $P < 0.05$).

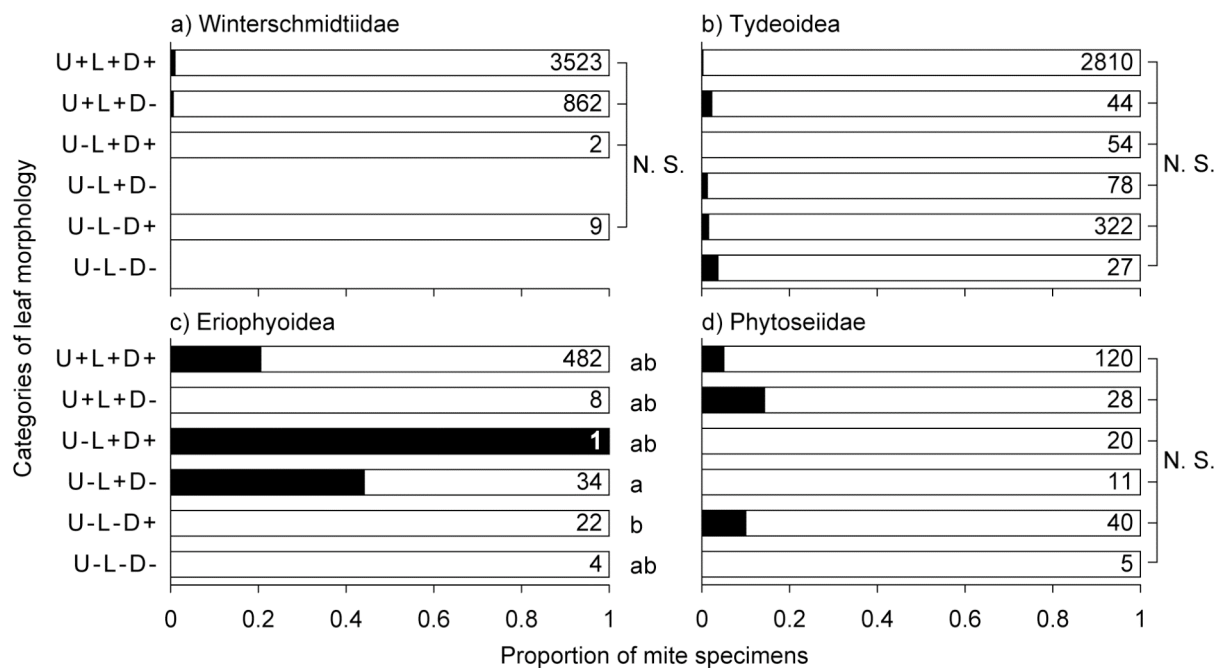


Fig. 1 Leaf-surface distributions of four major mite taxa on plants categorized by foliar fine structures, showing the proportions of mite individuals collected from adaxial (filled bars) and abaxial (open bars) leaf surfaces and total numbers (adaxial + abaxial) of collected mites (numerals in the bars). Unshared letters indicate a significant difference at $P < 0.05$ by 2×4 (Winterschmidtidae) or 2×6 (other three taxa) Fisher's exact tests with Holm–Bonferroni correction. Morphology category coding is as follows: presence (+) or absence (–) of hairs on the upper (U) or lower surface (L), and of leaf domatia (D).

The saturated log-linear model, which contained all three-way and two-way interactions, was supported by AIC (i.e., the

minimum value of the information criterion), whereas the model that contained two two-way interactions – “leaf × mite” and “mite × side” – was supported by the BIC (Table 2). The difference between the lowest AIC (saturated model) and the second lowest AIC (i.e., the model supported by the BIC) was only 2.54 (Table 2). This shows that only interactions of “leaf × mite” and “mite × side” are supportable. Support for “leaf × side” and the three-way interaction “leaf × mite × side” was rather marginal. Hence, it appeared that taxon composition of foliar acarine community differed with leaf surface morphology and adaxial–abaxial distribution patterns were different among mite taxa to some extent, whereas the adaxial–abaxial distribution pattern of each mite on each host plant did not differ drastically with leaf surface morphology.

Seasonal change in intra-leaf distribution of mite taxa on VEP

In the 2007–2008 survey on VEP, we collected 41,758 mite individuals (another 45 unidentified specimens were not included) belonging to 11 different taxa from 1,836 leaves (Fig. 2), of which 41,181 individuals (98.6%) (95% CI: 98.5–98.7%) were collected from the abaxial side. Winterschmidtidae, Tydeoidea, Eriophyoidea, and Phytoseiidae were the four most abundant taxa, accounting for 55.9, 37.2, 3.5, and 3.0%, respectively, of all collected mites from VEP; collectively they accounted for 99.6% of all identified mite specimens. All mounted specimens of Winterschmidtidae from VEP were identified as *Czenspinskia lordi* Nesbitt (Sudo et al. 2010). The tydeoid and eriophyoid mites were likely to be composed of several distinct species, but species were not identified. Of the phytoseiid mites five species were identified: *Paraphytoseius urumanus* (Ehara), *Phytoseius (Dubininellus) blakistoni* Ehara, *P. (D.) nipponicus* Ehara, *P. (D.) capitatus* Ehara, and one unknown *Phytoseius* species (Sudo et al. 2010).

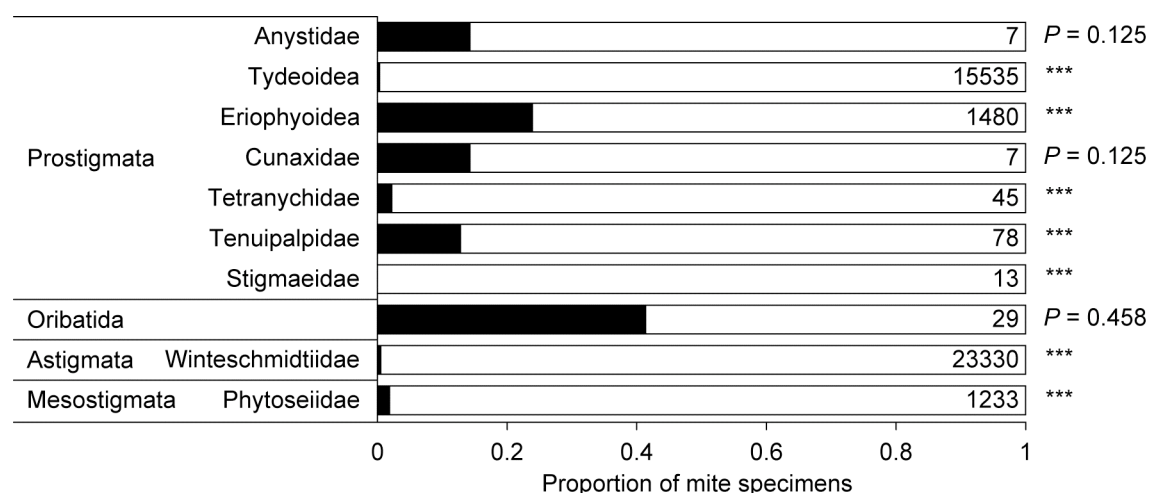


Fig. 2 Leaf-surface distributions of identified mite taxa on VEP showing the proportions of mite individuals collected from adaxial (filled bars) and abaxial (open bars) leaf surfaces and total numbers (adaxial + abaxial) of collected mites (numerals in the bars) in the 2-year survey at the two sites. The data on Ascidae (Mesostigmata: only one mite was found on VEP) are not shown. Asterisks shown at the right indicate significant differences (Exact binomial tests with null hypothesis of 1:1 adaxial–abaxial ratio, $P < 0.001$).

Seven of the 11 identified mite taxa were distributed in favor of abaxial leaf surfaces, after pooling all samples from all periods at both the Uryu-yama and Iwakura sites (Fig. 2; Exact binomial tests assuming even distribution, $P < 0.001$).

Individuals from abaxial leaf surfaces accounted for 99.5% (95% CI: 99.4–99.6%) in Winterschmidtidae, 99.7% (99.6–99.8%) in Tydeoidea, 76.1% (73.8–78.2%) in Eriophyoidea, and 98.1% (97.2–98.8%) in Phytoseiidae. In another four mite taxa that did not show a significantly biased distribution, Anystidae (seven mites in total), Cunaxidae (seven), and Ascidae (only one individual was obtained in the 2-year survey) were rare on VEP and tests for adaxial–abaxial distribution were not applied. Oribatid mites showed a relatively neutral distribution, more than any other mite taxa (Fig. 2).

At Uryu-yama, AIC supported the model with all three of the two-way interactions – “season \times mite”, “mite \times side” and “season \times side” – while the BIC supported the model that contained two two-way interactions – “season \times mite” and “mite \times side” – and lacked “season \times side” (Table 3). At Iwakura, the model that contained all three of the two-way interactions was supported by both AIC and the BIC (Table 3). This suggests that the acarofauna on VEP changes with sampling periods (seasonal fluctuation patterns of populations differ among mite taxa), and different mite taxa on VEP have different adaxial–abaxial distributions, while the seasonal change in the adaxial–abaxial distribution of the whole foliar mite community is dependent on circumstances. Perhaps the partial support for the “season \times side” interaction does not imply seasonal change of the adaxial–abaxial distribution in each mite taxon. It likely came from both of the difference in the baseline adaxial–abaxial ratios and the difference in seasonal population fluctuation patterns between mite taxa on VEP. Eriophyoidea, which has a relatively high proportion of adaxial individuals compared to the other three major taxa, disappeared in summer while the other three taxa occurred continuously from spring to autumn.

In the four major mite taxa on VEP, three – Winterschmidtidae, Tydeoidea, and Phytoseiidae – showed abaxial-biased distribution throughout the seasons. In 28 samples (14 months \times 2 sites), the ratio of individuals collected from the abaxial surfaces of VEP leaves ranged from 97.1 to 99.5 to 100% (min–median–max) in Winterschmidtidae, 97.7 to 99.7 to 100% in Tydeoidea, and 78.6 to 99.0 to 100% in Phytoseiidae. In contrast, the ratio of Eriophyoidea individuals collected from the abaxial side in each of the 12 samples (no eriophyoid mite was found in another 16) ranged from 23.8 to 82.6 to 100%. Eriophyoid mite populations on VEP clearly occurred twice a year; one peak was from late spring to early summer (May to July), another during autumn (October and November) (Sudo et al. 2010). The ratio of eriophyoid mite individuals on adaxial leaf surfaces was significantly higher in the autumn population (6.2% in spring vs. 36.1% in autumn; Fisher’s exact test, $P < 0.001$).

In Phytoseiidae, the adaxial–abaxial ratio of eggs collected from VEP leaves did not differ significantly from the ratio of the motile stage (Fisher’s exact test, $P = 0.72$, $n = 118$ eggs; 99.2% of eggs and 98.1% of motile forms were from the abaxial side). In contrast, in Tenuipalpidae, the ratio of individuals collected from adaxial leaf surfaces was significantly higher in eggs than in the motile stage (Fisher’s exact test, $P < 0.01$, $n = 62$ eggs; 35% of eggs and 13% of motile forms were from the adaxial side).

Diel dynamics of intra-leaf mite distribution on VEP

In the VEP sampling on 31 October 2009, we collected 540 mites from 54 leaves in daytime and 303 individuals from 54 leaves in nighttime (843 mites in total). Winterschmidtidae (318 mites), Tydeoidea (237), Phytoseiidae (236), and Tenuipalpidae (41) accounted for 37.7, 28.1, 28.0, and 4.9% of all collected mite specimens, respectively. We found only one eriophyoid individual. Another 17 mite individuals (8 winterschmidtids, 5 phytoseiids, and 4 unidentified individuals) were found in media, which were not included in the analyses. Total numbers of collected mites in day and night samplings, respectively, were 237 and 81 for Winterschmidtidae, 142 and 95 for Tydeoidea, and 121 and 115 for Phytoseiidae (Fig. 3).

As for the adaxial–abaxial distribution (mite individuals from adaxial leaf surfaces vs. mites from both the abaxial leaf blades and domatia), Winterschmidtidae and Phytoseiidae did not show diurnal change, whereas the ratio of Tydeoidea on the upper surfaces increased significantly during nighttime (Fig. 3). Within the abaxial side of a VEP leaf, the distribution between leaf blade and domatia differed diurnally in some mite taxa. Winterschmidtidae did not show diurnal change, whereas the ratio of tydeoid mites found inside domatia decreased at night (Fig. 3). In contrast, the ratio of phytoseiids inside domatia increased at night (Fig. 3). All individuals of phytoseiids collected inside domatia were larvae or protonymphs. The adaxial–abaxial distributions of individuals on VEP in the same place (Iwakura) and during October and November 2008 (daytime data) were 6:768 (adaxial: abaxial) for Winterschmidtidae, 3:882 for Tydeoidea, and 2:205 for Phytoseiidae, which did not significantly differ from the 2009 daytime sampling data (0:237 for Winterschmidtidae, 1:141 for Tydeoidea, and 1:120 for Phytoseiidae; 2×2 Fisher's exact tests, $P > 0.05$) (Fig. 3).

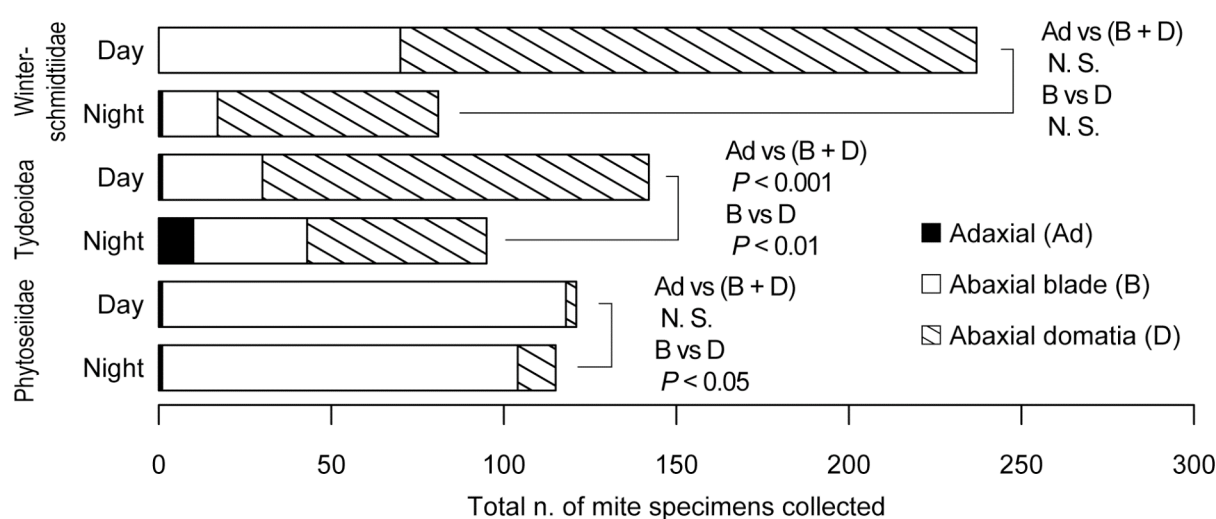


Fig. 3 Intra-leaf distribution of major mite taxa on VEP under day and night conditions of the sampling in Iwakura on 31 October 2009, showing the proportions of mite individuals collected from adaxial (filled bars) or abaxial (open bars) leaf blades and domatia (shaded bars). P values shown at the right are by 2×2 Fisher's exact tests.

Discussion

Generally, abaxial leaf surfaces of trees were more hairy and abundant in pollen, an alternative food resource for phytoseiid mites, rather than adaxial surface (Kreiter et al. 2002). In addition, a leaf domatium is an organ generally formed on abaxial leaf surfaces with a few exceptions (O'Dowd and Willson 1989) and exploited by mites as refuge from low humidity (Grostal and O'Dowd 1994) and/or predators (Norton et al. 2001). Among the tree and shrub species in forest ecosystems, availability of domatia and dense hairs on a leaf is positively correlated with population density of some fungivorous (e.g. Winterschmidtidae or Tydeoidea) and predaceous (e.g. Phytoseiidae) mite taxa (Duso 1992; O'Dowd and Pemberton 1998; Sudo et al. 2010). Thus, the adaxial–abaxial heterogeneity in leaf-surface morphology may explain the abaxial biased distribution in Winterschmidtidae, Tydeoidea and Phytoseiidae on VEP. Whereas the ratio of tydeoids on the adaxial leaf surfaces significantly rose at night, the majority of biomass in foliar mite assemblage on VEP remained on the abaxial leaf surfaces throughout seasons and both during daytime and night.

Although the architecture of leaf surfaces and resultant resource availability may contribute to the abaxial biased distribution of plant mites, we found that mite communities were concentrated on abaxial rather than adaxial leaf surfaces, not only on trees with dense trichomes and/or domatia but also on those with glabrous leaves. This implies that the physical (i.e., abiotic) environment might be one factor that accounts for the distribution of mites between adaxial and abaxial leaf surfaces. However, we found that the false spider mite (Tenuipalpidae: *Brevipalpus obovatus*), Eriophyoidea, and an oribatid mite (Oribatida) exploited not only abaxial leaf surfaces but also adaxial leaf surfaces.

For the false spider mites (*B. obovatus*), the ratio of eggs collected from the adaxial leaf surface to eggs collected from the abaxial surface, was significantly higher than the ratio of motile individuals. Although diurnal dynamics within host plants were known in some phytoseiid mites (Onzo et al. 2003, 2009; cf. Villanueva and Childers 2005), we found that both eggs and motile forms of Phytoseiidae were rare on adaxial surfaces in all seasons and during both day and night. Preference of mites for abaxial leaf surface had been explained as a result of adaptation to avoid wind and rainfall (Jeppson 1975). However, recent studies demonstrated that solar UVB radiation also largely affected the behavior of spider mites (Ohtsuka and Osakabe 2009; Sakai and Osakabe 2010) and phytoseiid mites (Onzo et al. 2010), and possibly restricted their foliar surface availability. Since *Phytoseius nipponicus* Ehara was the phytoseiid mite species commonly observed on VEP (Sudo et al. 2010) and that preyed upon *B. obovatus* eggs (M. Sudo, unpublished data), it is likely that *P. nipponicus* was also vulnerable to UVB radiation as well as other phytoseiid mites.

Therefore, oviposition on the upper leaf surfaces might provide a benefit for *B. obovatus* to decrease predation risk if their eggs are invulnerable to solar UVB radiation. The benefit similar to that of *B. obovatus* might be expected for Eriophyoidea, which was a potential prey for Type III generalist predators of the Phytoseiidae, such as *P. nipponicus* (McMurtry and Croft 1997). Meanwhile, population density of the eriophyoid mites on VEP reached peaks in spring and autumn, whereas it disappeared in summer (Sudo et al. 2010). Because solar UVB radiation and temperature increases from spring to summer and decreases from summer to autumn (winter) in Japan, the occurrence of eriophyoid mites might largely depend on such physical environmental factors. In addition, the upper leaf surface is thought to offer eriophyid mites a better location than the lower leaf surface to initiate takeoff behavior for aerial dispersal because of the greater exposure to air currents (Fournier et al. 2004). Oribatid mites have their center of diversity in soil ecosystems and on tree bark (Watanabe 1997; Walter and Behan-Pelletier 1999). They are characterized by armor (solid, pigmented exoskeleton) (Walter and Proctor 1999), which might work as protection against some harsh environments experienced on the upper leaf surfaces.

Therefore, the possibility of habitat differentiation in adaxial–abaxial leaf surface distribution in some mite taxa and its cost and benefit, especially in relation with their predators, should be investigated in the future. Acquisition of a mechanism that mitigates environmental stresses such as UVB is essential for plant mites to colonize the upper leaf surfaces, and some studies have claimed the potential role of pigmentation with carotenoid or melatonin (Suzuki et al. 2008, 2009) in UV tolerance in spider mites. Jeppson (1975) has mentioned several spider mite species, belonging to the genera *Bryobia*, *Panonychus* and *Oligonychus*, exploit upper (adaxial) leaf surfaces of their host plants. Interspecific comparison of pigmentation and UV tolerance among various mite taxa or trophic levels, when combined with further investigation on their leaf-surface preference, may increase our understanding of the spatiotemporal segregation of habitat in foliar acarine communities.

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